| **Tobramycin** |
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| **Purpose** | This procedure provides instructions for performing TOBRAMYCIN on ABBOTT INSTRUMENTATION. The Alinity c Tobramycin assay is used for the quantitative determination of tobramycin in human serum or plasma on the Alinity c analyzer. Tobramycin is also referred to as Tobra. |
| **Policy Statements** | This procedure applies to all personnel responsible for operating the Alinity c at Children’s Minnesota Laboratory, Minneapolis. |
| **Principle** | The Alinity c Tobramycin assay is a homogeneous particle-enhanced turbidimetric inhibition immunoassay (PETINIA). The assay is based on competition between drug in the sample and drug coated onto a microparticle for antibody binding sites of the tobramycin antibody reagent. The tobramycin-coated microparticle reagent is rapidly agglutinated in the presence of the anti-tobramycin antibody reagent and in the absence of any competing drug in the sample. The rate of absorbance change is measured photometrically and is directly proportional to the rate of agglutination of the particles. When a sample containing tobramycin is added, the agglutination reaction is partially inhibited, slowing down the rate of absorbance change. A concentration-dependent classic agglutination inhibition curve can be obtained, with maximum rate of agglutination at the lowest tobramycin concentration and the lowest agglutination rate at the highest tobramycin concentration.**Methodology:** Particle-enhanced turbidimetric inhibition immunoassay (PETINIA)For additional information on system and assay technology, refer to the Alinity ci-series Operations Manual, Section 3. |
| **Clinical Significance** | Tobramycin sulfate is an aminoglycoside derived from *Streptomyces tenebrarius*. This aminoglycoside antibiotic is used to treat seriousbacterial infections by inhibiting the growth of the bacterium byintervening in the protein synthesis thereby killing the bacterium.Tobramycin is absorbed minimally from the gastrointestinal tract.In the first 24 hours after intravenous dosing, the usual route ofadministration, about 99% of the tobramycin is excreted unchangedby the kidneys. The average half-life in patients with normal renalfunction is about 2 to 3 hours. Therapeutic serum levels varydepending on the microorganism involved and the patient’s toleranceto the drug. Tobramycin serum or plasma concentrations aremonitored to help guide therapy, since individual patient differencesrequire dose changes that are difficult to predict. Monitoringserum or plasma levels of tobramycin decreases the frequency ofserious toxic effects. |
| **Analyzer** | **Minneapolis: Abbott Alinity c (Sunquest method code: MACC)****BACKUP:** Abbott Alinity c (Sunquest method code: MALIC) |
| **Sunquest Test Codes** | **TOBR** |
| **Specimen** | Sample: Plasma or Serum (with or without gel barrier)**Preferred:** Lithium Heparin**Alternative:** SST, Sodium Heparin, K2-EDTA, K3-EDTA**Suggested Patient Preparation:** Samples for the Alinity c Tobramycin assay should be drawn just prior to a dose (trough level) to confirm that an adequate dose has been prescribed. Peak specimen should be drawn 30 minutes after a 30 minute IV infusion.**Minimum sample volume:** 0.6 mL blood, 0.2 mL serum/plasma**Stability when separated from cells/gel:** **20 to 25°C:** Not specified; 8 hours\***2 to 8°C:** 7 days**-20°C:** 14 days **\*NOTE:** Samples containing carbenicillin or piperacillin should be stored frozen if a delay in analysis of more than 8 hours is anticipated. Failure to freeze samples containing these antibiotics may result in falsely low tobramycin levels due to *in vitro* inactivation.**Rejection criteria:** Unlabeled tube, sample type other than serum or acceptable plasma**Preparation:** 1. Whole blood specimens should be centrifuged following complete clot formation, according to Specimen Processing procedures prior to analysis.
2. Serum or plasma should be physically separated from cells as soon as possible with a maximum limit of two hours from the time of collection.
3. Specimens should be free of particulate matter.
4. Transfer serum or plasma directly to a properly labeled pilot tube.
5. Architect and Alinity systems utilize a specimen level detect mechanism, so special racks specific to tube-type are not required.
6. Minimum labeling includes sample accession ID, and/ or patient name, medical record number, collection date and time.
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| **Reagents** | **Reagent Handling** Reagents are shipped refrigerated or on cold gel packs. Upon receipt, place reagent cartridges in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate. If a reagent cartridge is dropped, place in an upright position for 8 hour before use to allow bubbles that may have formed to dissipate. **Immediately prior to loading on the analyzer, gently invert the cartridge 5 times. Check to ensure there are no bubbles.**Reagents are susceptible to the formation of foam and bubbles. Bubbles may interfere with the detection of the reagent level in the cartridge and cause insufficient reagent aspiration that may adversely affect results. Use a pipette to remove all bubbles prior to loading on the Alinity or Architect system.* Do not use reagents beyond the expiration date.
* Do not pool reagents within a kit or between kits.
* Do not use components from one lot with components from another lot.
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| ***Product Description*** | ***Product Code*** | ***Stability*** |
| Abbott Alinity c Tobramycin Reagent Kit  | 09P9020 | **Store at:** 2 to 8°C **Unopened:** Until manufacturer’s printed expiration date**On-board**: 32 days |
| Abbott Alinity c Tobramycin Calibrators  | 09P9001 | **Store at:** 2 to 8°C**Unopened:** Until manufacturer’s printed expiration date**Opened expiration:** Until manufacturer’s printed expiration date (Store tightly capped with new replacement cap). |

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| **Risk and Safety** | **CAUTION:** This product contains human-sourced and/or potentially infectious components. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all humansourced materials should be considered potentially infectious. It is recommended that these reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents. The human-sourced material used in **R2** is nonreactive for HBsAg, HCV, HIV-1, and HIV-2.The following warnings and precautions apply to: **R1**Contains tris hydroxymethyl aminomethane and sodium azide. Causes mild skin irritation. Contact with acids liberates very toxic gas.The following warnings and precautions apply to: **CAL**  Contains sodium azide. Contact with acids liberates very toxic gas.Special disposal not indicated.Safety data sheets (MSDS/SDS) available on [Children’s Intranet](https://starnet.childrenshc.org/emergency-and-safety/) |
| **Calibration** |

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| Assay Range: | 0.3 to 10.0 µg/mL |
| Reference Material: | Abbott Alinity c Tobramycin Calibrators |
| Suggested Calibration Levels: | Approximate values:CAL 1: 0.0CAL 2: 0.5CAL 3: 1.5CAL 4: 3.0CAL 5: 6.0CAL 6: 10.0 |
| Calibration Scheme: | 6 Levels,  |
| Calibration Frequency: | 7 Days, and with every new lot. This assay may require recalibration after maintenance to critical parts or subsystems or after service procedures have been performed. |
| AMR | AMR is verified with every calibration. |

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| **Quality Control** | **QC Material:** Bio-Rad Liquichek™ Therapeutic Drug Monitoring Control Levels 1 and 3**Frequency:** Two levels each day of use**Stability:** Stable until the expiration date when stored frozen between -20 and -40°C. Once thawed, opened, and stored tightly capped at 2 to 8°C, this product is stable for 5 days in Minneapolis (due to estradiol in this control). **Preparation**: This product should be treated the same as patient specimens and run in accordance with the instructions accompanying the instrument, kit, or reagent being used. * To thaw the product, allow it to stand at room temperature (18° to 25°C) until completely thawed but no longer than one (1) hour.
* After thawing, the product **MUST** be gently swirled and inverted several times to ensure homogeneity.
* For optimal analyte stability in the thawed state, promptly return to 2 to 8°C storage after each use and minimize the time at room temperature to no more than 20 minutes daily.
* **Before each use**, gently swirl the contents until homogeneous with no visible signs of precipitate.

**Acceptable ranges:** * Non-Bio-Rad controls will utilize manufacturer ranges and 2 SD Westgard rules.
* New lots of Bio-Rad controls should be run for 20 days in parallel with the current lot whenever possible prior to switching to the new lot.
* Refer to the Westgard Rules in Chemistry procedure for current Westgard rules in place for each analyte.
* **Acceptable ranges are current in Unity Real Time only.** Quality Control results must be rejected in Sunquest when the results cross the interface.
* In the event of a QC failure, refer to the [Unity Real Time QC Review, General User](https://starnet.childrenshc.org/References/labsop/chem/quality/ch-2.17-unity-real-time-qc-review-general-user.pdf) and navigate to the QC Troubleshooting section.
* Do not load or release patients until QC is acceptable in Unity Real Time.
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| **Interferences** | **Hemolysis, Icterus & Lipemia (HIL) Index Values:**

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At HIL levels at or above the specified cutoff value, append the appropriate comment AFTER visually confirming presence of interferent: -HP for “Hemolysis present, may affect results.” -BIN for “Bilirubin Interference”-LINT for “Lipid Interference” No endogenous substance interference noted in Abbott studies.  |
| **Reference Intervals** | **Peak Levels:** 4-8 µg/mL **Peak Toxic Levels:** > 12 µg/mL **Trough**: 0-4 µg/mL **Trough Toxic Levels** >4 µg/mL Samples >8 µg/mL will be flagged as abnormal for all ages in Sunquest.The susceptibility of the infecting organism, the severity of the infection, and the general health of the patient should be considered when determining an adequate drug level for patients. |
| **Critical Values** | **> 10 µg/mL**Critical results are called and documented according to the Critical Values policy. |
| **Limitations** | Samples containing carbenicillin or piperacillin should be stored frozen if a delay in analysis of more than 8 hours is anticipated. Failure to freeze samples containing these antibiotics may result in falsely low tobramycin levels due to in vitro inactivation.For diagnostic purposes, the test findings should always be assessed in conjunction with the patient’s medical history, clinical examinations, and other findings.In very rare cases, patient samples may contain heterophile antibodies, which may produce low results with the Alinity c Tobramycin assay. Interfering heterophile antibodies occur at a low frequency in the general population. These antibodies can cause autoagglutination of the microparticle reagent leading to undetected erroneously low results.Patient samples which contain the drugs amikacin, kanamycin A, kanamycin B, and/or 3',4'-dideoxykanamycin B may yield falsely elevated values for tobramycin. Refer to the Cross-Reactants section of the Instructions for Use (package insert) for further explanation. However, these drugs are not usually coadministered with tobramycin. High concentrations of penicillins or cephalosporins have been shown to inactivate tobramycin in vitro. The degree of inactivation is dependent on the particular aminoglycoside being measured, the type and concentration of the penicillin or cephalosporin that is also present, and the storage conditions of the sample. Samples from patients receiving additional antibiotics of these types should be assayed immediately or stored frozen. |
| **Dilutions** |

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| Max Auto Dilution: | None |
| Maximum Manual Dilution: | 1:2 |
| Diluent: | Abbott Alinity c Tobramycin Calibrator Level 1 |
| Manual Dilution: | Follow Abbott [Alinity Operator’s Manual](https://starnet.childrenshc.org/References/labsop/chem/operator/alinity-ci-series-operations-manual.pdf) instructions for programming automated dilutions. The system will automatically calculate the concentration of the sample and report the result. If a diluted sample result is less than the lower value of the measuring interval of 0.3, do not report the result. Rerun and/or investigate for other possible causes of error. |

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| **Result Reporting** | * Results between 0.3 and 8.0 µg/mL without error messages are released
* Results below 0.3 µg/mL without error messages are reported as < 0.3 µg/mL
* Results >10.0 µg/mL should be diluted manually 1:2 with Tobra Calibrator 1. Results without error flags following this dilution are reported according to critical values policy.
* Results > 20.0 following manual dilution are reported as > 20.0 µg/mL
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| **Specimen Storage** | Promptly stopper tested specimen and store upright in a specimen rack. Every 8 hours remove specimens to refrigerator/freezer storage. Samples are retained 14 days in specimen storage freezer. |
| **References** | 1. Abbott Alinity c Tobramycin Reagent Kit Instructions for Use, Abbott Diagnostics Division, Abbott Park, IL USA. Revised October 2018
2. Abbott Alinity c Tobramycin Calibrator Kit Package Insert, Abbott Diagnostics Division, Abbott Park, IL USA. Revised March 2018
3. Bio-Rad Liquichek Immunoassay Plus Package Insert, Bio-Rad Laboratories, Irvine CA USA.
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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
|  | Elauteria Earnhardt | April 24, 2020 | New Procedure for Abbott analyzers |
| 1 | Erin Bartos | October 28, 2020 | Formatting, added assay number/title, dilutions, AMR, calibrations, reference interval, references, etc for new analyzer |